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Susceptibility of Normal and Transformed Cell Lines to Cytostatic and Cytocidal Effects Exerted by Macrophages^{1, 2}

R. Keller^{3, 4}

SUMMARY—Activated, nonimmune macrophages exerted profound effects on the proliferation and viability of eukaryotic target cells in vitro. Pronounced macrophage-mediated cytostasis was exerted on every rapidly proliferating cell line examined, irrespective of transformation, species derivation, cell type, or growth characteristics. However, the magnitude of cytostasis effected differed markedly among the 40 cell lines tested. There was no evident correlation between susceptibility to cytostasis and degree of transformation. Transformed cell lines with high and with low malignant attributes were affected equally. A comparable pattern was discerned for cytotoxic effects of macrophages, in which the susceptibility of transformed targets was independent of the degree of malignancy.—*J Natl Cancer Inst* 56: 369–374, 1976.

Recent work (1–5) has shown that apart from the important function of mononuclear phagocytes in pathologic processes, the presentation of antigen to immunocompetent cells, and resistance against intracellular microorganisms, mononuclear phagocytes also exert profound effects on eukaryotic cells. These in vitro studies have yielded voluminous data indicating that activated, nonimmune macrophages (AM) can affect target cells in a variety of ways, and that they can discriminate between normal and transformed cells and resting and replicating cells. These destructive capabilities apparently involve the metabolic state of a macrophage population, as well as the proportion of effectors to targets. The findings thus far indicated that the cytostatic effects on targets exerted by AM are independent of target species (syngeneic, allogeneic, or xenogeneic), target cell type (epithelial or lymphoid), target growth characteristics (monolayer or suspension), or transformation (normal vs. neoplastic tissue) (4–7). There is general agreement (1–4, 8–10) that interaction of AM with tumor targets results in an altered morphology and decrease in the number of cells, attesting to the AM's capacity to kill tumor targets in vitro. However, further investigation on many cell lines derived from normal and transformed tissue disclosed that macrophage cytotoxic target cell effects are not invariably correlated with the malignant attributes of cell lines derived from transformed tissue.^{5, 6} The present work further explores whether the macrophage-mediated cytotoxic effect is selective for tumors.

MATERIALS AND METHODS

Target cell lines.—Rat: Cells derived from normal rat kidney (NRK) and rat kidney cells infected with B₇₇ Rauscher murine leukemia virus (B₇₇ = NRK) (11), a gift from Dr. T. Graf, were grown in Eagle's minimum essential medium (MEM) (12) modified as follows: 280 mg glutamine/liter, 100 mg calcium/liter, 1 g NaHCO₃/liter, 2 g glucose/liter, and 1 mg biotin/liter, and supplemented with 100 U penicillin/ml, 50 µ streptomycin/ml (modified MEM), 10% fetal calf serum (FCS), and 10% tryptose phosphate (Difco Laboratories, Detroit, Mich.). DA rat tumors were those described in (4) or were newly induced with polyoma virus, dimethylbenz[*a*]anthracene

(DMBA), or 3-methylcholanthrene (MCA). These tumor cells were grown in modified MEM supplemented with 10% newborn calf serum (NCS). DA rat adult and embryo fibroblasts were repeatedly newly established (6) and grown in modified MEM supplemented with 10% FCS.

Mouse: The A9 cell (having little ability to grow progressively in vivo), its highly malignant counterpart A9HT (high incidence of take) (13), and the hybrid cell lines between these L-cell derivatives and malignant mouse tumors, such as Ehrlich (spontaneous carcinoma), SEWA (polyoma-induced sarcoma), MSWBS (MCA-induced sarcoma), and YACIR (an immunoresistant derivative of the YAC tumor, a Moloney virus-induced lymphoma) (13, 14), provided by Dr. George Klein, were grown in nonmodified Eagle's MEM. BALB/c3T3 cells and BALB/c simian virus 40 (SV40)-transformed 3T3 cells, originally obtained from Dr. Stuart Aaronson, were maintained in modified MEM with 10% FCS. Suspension cultures of the transplantable murine mast cell tumor P-815 X2, obtained from Drs. R. Schindler and M. Bertschmann, were grown as described in (15); the medium was supplemented with 10% NCS instead of horse serum.

Human: Three adherent cell lines derived from human mammary carcinomas were used: BT-20, supplied by Dr. J. Fogh, was grown in modified MEM supplemented with 10% FCS; MPZ-2 and MPZ-4 were freshly established from a biopsy of human breast cancer, provided by Dr. J. R. Rüttner and grown in modified MEM supplemented with 15% FCS. Two human melanoma lines were used. SK-melanoma-1 (MEL-1) cells, obtained from Dr. K. T. Brunner, were grown in suspension in modified MEM supplemented with 10% FCS. Melanoma cells (RPMI 7932), provided by Dr. W. D. Terry, were grown in adherent culture in modified MEM with 10% FCS. SK-OS-5, derived from an osteogenic sarcoma and supplied by Dr. J. Fogh, was grown in adherent culture

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⁵ Bregnard A, Gehring WJ, Keller R, et al: In preparation.

⁶ Keller R: Unpublished data.

in modified MEM with 10% FCS. The Burkitt's lymphoma cell line RAJI, obtained from Dr. G. Klein, was grown in modified MEM supplemented with 10% FCS. BEN, a tumor cell line producing carcinoembryonic antigen, was supplied by Dr. Ch. Sauter and grown in adherent culture in modified MEM with 10% FCS. Cell lines were repeatedly checked for the possibility of mycoplasma contamination, but none was found.

Origin, culture conditions, and properties of other cell lines used were described in (6).

Macrophage monolayers were prepared as described in (4, 6). Peptone-induced peritoneal cells from inbred DA rats were seeded into plastic petri dishes. After 30 minutes at 37° C, the nonadherent cells were removed by intensive washing. After this procedure, at least 96% of the cells in the monolayer of approximately 2×10^6 cells showed the characteristics of macrophages. To these macrophage monolayers, target cells (2×10^5 /dish) were immediately added and the cultures were maintained at the appropriate temperature in a humid atmosphere of 5% CO₂ and 95% air.

Measurement of cytostasis.—Residual target cell proliferation was assessed after varying intervals of macrophage–target cell interaction by: 1) exposure for 60 minutes at 37° C to 1 μ Ci ³H-methylthymidine (³H-TDR)/dish (5,000 mCi/mmol; The Radiochemical Centre, Amersham, Buckinghamshire, England), and processing as described in (4, 6); or 2) by exposure for 8 hours at 37° C to 0.1 μ Ci ¹²⁵I-5-iodo-2'-deoxyuridine (¹²⁵IUDR, sp act, 8–10 μ Ci/ μ g; The Radiochemical Centre). After careful washing, the cells were washed twice with 1.5% perchloric acid and radioactivity was measured in an automatic gamma counter (Tracerlab, Inc., Waltham, Mass.).

Assessment of target cell viability.—Two methods were used to assess target cell viability. In experiments performed to assess the capacity of target cells to reestablish growth, 2×10^5 targets were cultured in the presence of 2×10^6 AM (6). After 72 hours, the cells were harvested by trypsinization, washed, counted, and diluted to a concentration of 300/3.5 ml; aliquots of this volume were also dispensed to 30-ml Falcon culture flasks. After 10 days' incubation in 5% CO₂ at 37° C, we assessed target cell proliferation by adding 1 μ Ci ³H-TDR for 60 minutes at 37° C and by processing the cells as described. Lines derived from the A9 cell were easily distinguished morphologically from AM. Thus the number of target cells remaining in culture after a 72-hour interaction with AM could be counted after trypsinization.

In other experiments, the release of ¹²⁵IUDR from prelabeled targets was a measure of macrophage-mediated target cell damage. Subconfluent cultures of target cells grown in 250-ml Falcon tissue culture flasks containing 10 ml medium were pulsed for 8 hours with 1 μ Ci ¹²⁵IUDR/flask (0.1 μ Ci/ml) in the presence of 2'-fluoro-5-deoxyuridine (FUDR) at a concentration of 10^{-5} M. The FUDR, a known inhibitor of thymidylate synthetase, was included to increase ¹²⁵IUDR incorporation into DNA (16), in place of ³H-TDR.

In many target cell lines, incubation with a combination of ¹²⁵IUDR and FUDR in the concentrations and time indicated resulted in adequate specific labeling without marked signs of toxicity; these particular cell lines were used here. Following incubation with ¹²⁵IUDR for 8 hours, the cells were washed twice with phosphate-buffered saline to remove unincorporated isotope. The prelabeled cells (2×10^5 /dish) were then added to macrophage monolayers and incubated for varying intervals,

after which the cells were removed by a pipette and then centrifuged. Supernatants and cells were enumerated separately in an automatic Tracerlab gamma counter. Results are expressed as a percent of cytotoxic capacity, calculated as follows:

$$\frac{\text{Experimental dpm} - \text{control dpm}}{\text{total dpm}} \times 100,$$

where dpm = disintegrations per minute.

RESULTS

Cytostatic Target Cell Effects Mediated by Macrophages

Following interaction for different intervals with 10 macrophages per target cell, the ³H-TDR incorporation, a measure of residual target cell proliferative capacity, was profoundly diminished in all of the target cell lines examined (table 1). However, there were considerable differences in the susceptibility of different target cell lines to macrophage-mediated cytostasis. Proliferation of several cell lines (i.e., polyoma virus-induced syngeneic rat tumor cells, xenogeneic mouse SV40T3, and especially P-815 mastocytoma cells) was blocked even in the early phase of interaction and remained at a very low rate as interaction proceeded. Other cell lines were initially more resistant to macrophage-mediated cytostasis but were inhibited during the later phases of interaction; this was seen with 3T3, RPMI 7932, BEN, BT-20, MPZ-2, MPZ-4, SK-OS, and MEL-1 cells. Proliferation of RAJI and several other human lymphoblastoid cell lines was often enhanced in the early phase of interaction with macrophages, but was subsequently diminished as the interaction proceeded. DMBA- and MCA-induced syngeneic rat tumor cells, although effectively inhibited in the early phase, retained proliferative capacity during continuous interaction with macrophages. Proliferation of fibroblasts, derived from rat embryo (or adult) tissues and exposed to macrophages during in vitro passages 4–7 and 24–30, was distinctly diminished in the early phase but often remained unchanged or was even enhanced as interaction proceeded; such reversed reaction to macrophages was observed especially with recently explanted fibroblasts. Thus in all target cell lines examined, pro-

TABLE 1.—Marked inhibition of proliferation of normal and neoplastic target cells (2×10^5) in the presence of 2×10^6 DA rat macrophages

Target	Residual ³ H-TDR incorporation (% of control) after macrophage interaction for ^a		
	18 hr	36 hr	48 hr
Fibroblasts:			
Passages 4–7	52 (±48)	36 (±26)	37 (±24)
Passages 24–30	32 (±16)	29 (±11)	17 (±11)
Polyoma	23 (±5)	16 (±7)	14 (±7)
MCA	28 (±11)	19 (±16)	24 (±6)
DMBA	35 (±17)	28 (±13)	30 (±23)
3T3	53 (±20)	12 (±7)	9 (±6)
SV40T3	27 (±10)	10 (±6)	7 (±4)
P-815	3 (±3)	6 (±4)	7 (±6)
RAJI	123 (±42)	51 (±17)	36 (±14)
CLA-4	91 (±28)	23 (±9)	31 (±24)
MEL-1	83 (±32)	19 (±9)	16 (±9)
RPMI 7932	60 (±22)	7 (±2)	6 (±3)
BEN	53 (±29)	12 (±6)	13 (±6)
SK-OS	50 (±13)	15 (±6)	20 (±6)
BT-20	41 (±17)	9 (±5)	8 (±4)
MPZ-2	49 (±26)	17 (±8)	11 (±4)
MPZ-4	59 (±33)	17 (±5)	20 (±12)

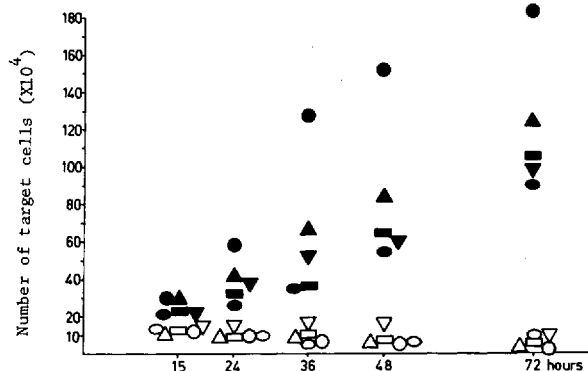
^a Values are means (±SD) of 10–20 experiments, each performed in triplicate.

liferation was clearly inhibited when the proportion of macrophages was in the majority, but the pattern of inhibition varied markedly from one cell line to another. Results similar to those with ^3H -TDR were obtained when targets, which interacted for varying intervals with macrophages, were pulse labeled with ^{125}I UDR.

Cytostatic and Cytocidal Macrophage Effects on Targets of Low and High Tumorigenicity

Enumeration of targets remaining after AM interaction of some lines revealed that, irrespective of whether targets were derived from normal or from transformed tissues, their number decreased as interaction proceeded (text-fig. 1). Since these experiments resolved neither the issue whether macrophage cytostatic and cytocidal effects were related nor whether cytocidal effects were indeed tumor specific, further experiments were made on other cell lines.

Cell lines derived from NRK, its virus-transformed counterpart, and from a temperature-sensitive mutant (*ts* 339) permissive at 33°C but not at 38°C (11), provided a useful model to probe the extent to which macrophage-mediated cytostatic and cytocidal effects are related to malignancy. Proliferation rate among these cells differed considerably, but was high enough in each instance to enable reliable quantitation of ^3H -TDR incorporation into target cell DNA (table 2). These data show that in every one of these cell lines the residual capacity to ^3H -TDR decreased as the period of interaction with AM increased. Moreover, the findings showed that NRK cells, although significantly inhibited, were less susceptible to macrophage-mediated cytostasis than were the transformed lines. However, the transformed



TEXT-FIGURE 1.—Decrease in number of neoplastic and normal target cells (initially 2×10^5) during interaction with macrophages (2×10^6). Target cell lines: \square = DMB-induced DA rat tumor cells; \triangle = polyoma-induced DA rat tumor cells; \circ = mouse SV40T3 cells; \diamond = mouse 3T3 fibroblasts; ∇ = CHO hamster fibroblasts. Closed symbols are targets alone; open symbols are targets in the presence of AM.

TABLE 2.—Correlation between degree of macrophage-mediated cytostasis and growth characteristics of different rat cell lines

Target ^a	Proliferative capacity (dpm at 60 hr)	Residual ^3H -TDR incorporation (% of control) by targets post culture with AM in ratio of 10 AM/target after interaction with AM for ^b					
		4 hr	12 hr	24 hr	36 hr	48 hr	60 hr
NRK	16,000–26,000	82 (± 21)	50 (± 15)	38 (± 12)	30 (± 8)	32 (± 3)	32 (± 5)
B ₇₇	34,000–88,000	63 (± 10)	31 (± 6)	14 (± 3)	11 (± 1)	11 (± 3)	10 (± 2)
<i>ts</i> , 38°C	48,000–120,000	52 (± 9)	16 (± 9)	8 (± 2)	8 (± 4)	8 (± 4)	8 (± 2)
<i>ts</i> , 33°C	82,000–212,000	40 (± 19)	13 (± 4)	8 (± 5)	10 (± 7)	5 (± 3)	4 (± 1)

^a B₇₇ = NRK cells infected with B₇₇ avian sarcoma virus; *ts* = NRK cells infected with temperature-sensitive mutant *ts* 339, permissive at 33°C and reverted to a normal phenotype at 38°C .

^b Values represent means (\pm SD) of 10 determinations.

TABLE 3.—Differing effects by AM on capacity of normal and transformed targets to reestablish growth

Target cell lines	Cloning efficacy ^a
NRK	53 (± 9)
B ₇₇	35 (± 6)
<i>ts</i> , 38°C	22 (± 9)
<i>ts</i> , 33°C	29 (± 6)

^a Percent of control as represented by the capacity for ^3H -TDR incorporation. Values are means (\pm SD) of 10 determinations.

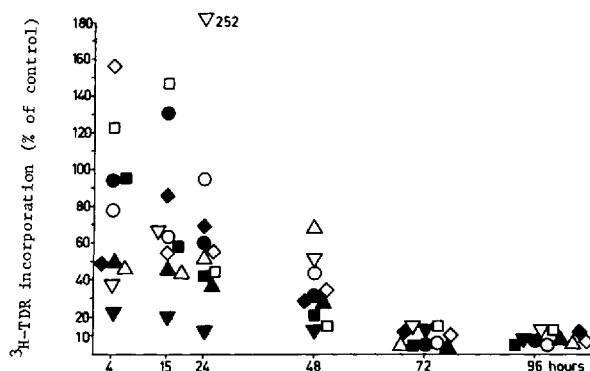
cells were equally affected, irrespective of whether or not they were permissive.

Experiments, in which loss of capacity to reestablish growth after prolonged culture with AM was taken as a measure of macrophage-mediated cytocidal target cell effects, revealed a significant cloning reduction in all cell lines examined (table 3). However, cytocidal effects were most pronounced in transformed cells, and no difference occurred between cells grown under permissive or nonpermissive conditions.

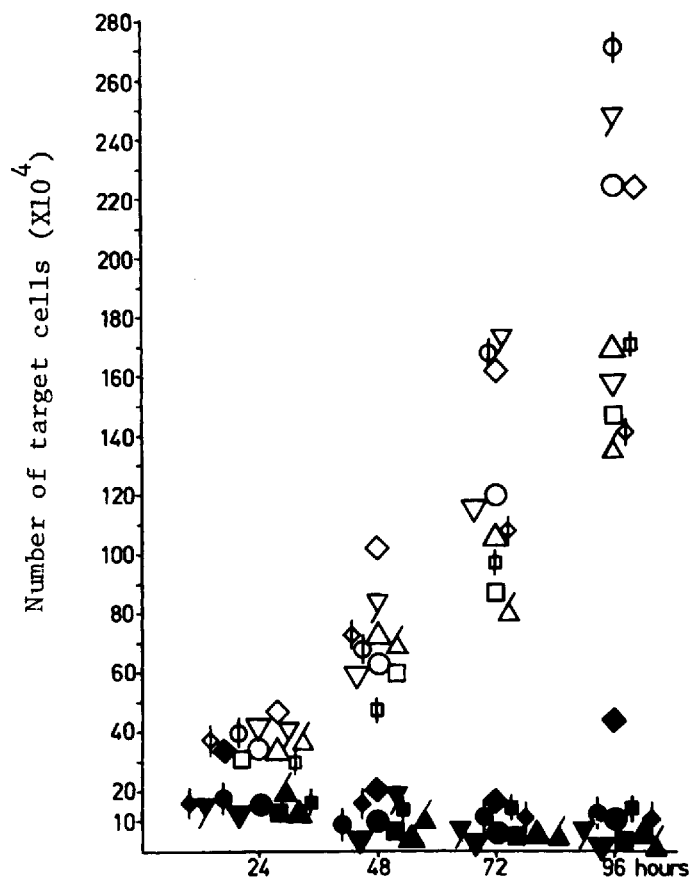
It was recently shown that the fusion of highly malignant mouse cells with normal cells or cells of low malignancy yields a hybrid with suppressed malignant characteristics (14). Among such hybrids, the A9 series is particularly interesting since hybrids with the slightly malignant L-cell subline showed suppressed malignancy, but partners of the highly malignant L-cell subline retained undiminished malignancy (13). Thus it appeared that a comparison between pairs of A9, A9HT, and the various A9-tumor hybrids might give information relevant to the present issue.

Accordingly, the macrophage effects on five paired lines consisting of a slightly malignant (A9) and highly malignant (A9HT) counterpart were assessed. The data in text-figure 2 show that in every cell line examined, interaction with AM resulted in a marked diminution of ^3H -TDR incorporation. However, when compared with most previously examined cell lines, several L-cell hybrids were surprisingly resistant to macrophage-mediated cytostasis; this became especially evident after 4 and 15 hours. After this interval, proliferation of a few cell lines was even stimulated by AM. Again, the susceptibility of the cell lines to macrophage-mediated cytostasis seemed independent of the degree of malignancy.

When macrophage-mediated cytocidal effects were assessed by an enumeration of the targets remaining after various intervals, all cell lines were comparably affected (text-fig. 3). In the absence of effectors, the cell numbers increased progressively; however, in their presence, cell numbers remained low and decreased as interaction proceeded (table 4). Again, no clear distinction was found in the susceptibility of cells with low or high malignancy within each comparable pair.



TEXT-FIGURE 2.—Macrophage-mediated (2×10^6) inhibition of target cell (2×10^5) proliferation is not dependent on degree of tumorigenicity. Target cell lines: ●=A9; ○=A9HT; ▼=A9/SEWA; ▽=A9HT/SEWA; ■=A9/MSWBS; □=A9HT/MSWBS; ▲=A9/YACIR; △=A9HT/YACIR; ◆=A9/EA; ◇=A9HT/EA. Closed symbols are slightly malignant derivatives; open symbols are highly malignant derivatives.



TEXT-FIGURE 3.—In pairs of L-cell derivatives with low (A9) and high (A9HT) tumorigenicity, the number of targets (initially 2×10^5) was similarly affected during interaction with 2×10^6 DA rat macrophages. Target cell lines: ○=A9; ▽=A9/SEWA; □=A9/MSWBS; △=A9/YACIR; ◇=A9/EA; ◊=A9HT; ▽=A9HT/SEWA; ◻=A9HT/MSWBS; △=A9HT/YACIR; ◊=A9HT/EA. Open symbols are targets alone; closed symbols are targets in the presence of AM.

Cytocidal Effects Mediated by Macrophages

Among the cell lines suitable for cytotoxic experiments (i.e., those which incorporated and subsequently retained an appropriate amount of $^{125}\text{IUDR}$), considerable differences were seen in label retention. Some cell lines, e.g., RAJI, RPMI 7932, SK-OS, or polyoma virus-induced rat

TABLE 4.—Effect of AM on capacity of pairs of targets of low and high tumorigenicity to reestablish growth

Tumorigenicity	Number of targets ^a
Low:	
A9	14
A9/MSWBS	4
A9/SEWA	2
A9/EA	14
A9/YACIR	7
High:	
A9HT	13
A9HT/MSWBS	12
A9HT/SEWA	9
A9HT/EA	11
A9HT/YACIR	1

^a Absolute No. of targets ($\times 10^4$; initially 2×10^5) remaining in culture after 96-hr interaction with AM.

tumor cells, retained a high proportion of the label for at least 48 hours, whereas others (especially MEL-1, BEN, BT-20, and rat fibroblasts) often showed considerable spontaneous release. This leakage is probably an expression of $^{125}\text{IUDR}$ - and/or FUDR-mediated toxicity. Although these differences in resistance to the labeling procedure may considerably affect the outcome of cytotoxic tests, the measurements of the label release from target cells proved reproducible and provided information. The results in table 5 show that macrophage-mediated release of $^{125}\text{IUDR}$ from prelabeled target cells was consistently observed after 18 hours; after an 8-hour interaction, no such release was detectable. In many cell lines, and most evident in those with low spontaneous release (RPMI 7932, SK-OS, and RAJI), specific release increased as interaction proceeded. However, other cell lines including P-815, MEL-1, 3T3, SV403T3, BT-20, BEN, MPZ-2, MPZ-4, and CLA-4, were also susceptible to cytotoxic macrophage effects. No difference was discerned between "normal" 3T3 fibroblasts and their virus-transformed counterparts. Among the cells examined, recently explanted syngeneic rat fibroblasts and DMBA-induced syngeneic rat tumors were resistant; cytotoxic effects were not consistently detectable.

A comparison of cytostatic and cytotoxic macrophage effects on these targets (tables 1, 5) indicates that the

TABLE 5.—Susceptibility of various target cell lines to macrophage-mediated cytotoxic effects

Cell line	Percent of cytotoxic activity effected ($^{125}\text{IUDR}$ release) at ^a		
	18 hr	36 hr	48 hr
Fibroblasts			
Passages 4-7	15 (± 14)	9 (± 7)	8 (± 7)
Passages 24-30	17 (± 8)	19 (± 12)	21 (± 9)
Polyoma	5 (± 5)	9 (± 6)	21 (± 10)
MCA	14 (± 16)	12 (± 9)	12 (± 8)
DMBA	9 (± 5)	9 (± 6)	8 (± 15)
3T3	13 (± 5)	9 (± 9)	10 (± 8)
SV403T3	16 (± 4)	23 (± 5)	16 (± 5)
P-815	20 (± 5)	28 (± 10)	28 (± 4)
RAJI	3 (± 3)	30 (± 7)	37 (± 7)
CLA-4	6 (± 4)	17 (± 9)	18 (± 7)
MEL-1	23 (± 8)	38 (± 6)	45 (± 5)
RPMI 7932	11 (± 4)	44 (± 10)	50 (± 7)
BEN	3 (± 3)	10 (± 10)	13 (± 17)
SK-OS	23 (± 4)	48 (± 10)	48 (± 7)
BT-20	20 (± 6)	49 (± 17)	59 (± 6)
MPZ-2	30 (± 11)	28 (± 11)	26 (± 6)
MPZ-4	9 (± 6)	12 (± 7)	12 (± 6)

^a Values are means (\pm SD) of 10-15 experiments, each performed in triplicate.

changes in these parameters during interaction were not consistently parallel. A close parallelism in the degree and time course of cytostatic and cytotoxic manifestations was especially notable in the lymphoblastoid lines RAJI and CLA-4 and in rat fibroblasts. However, no such parallelism in cytostatic and cytotoxic effects was seen in polyoma virus-induced DA rat tumor and P-815 mouse mastocytoma cells.

DISCUSSION

Prior *in vitro* studies (4, 6) in this laboratory consistently demonstrated that interaction of macrophages with targets first affects target cell proliferation and is often accompanied by a decrease in the number of targets; the capability of remaining targets to reestablish growth was diminished. These studies led to the provisional conclusion that macrophage-mediated cytostasis transcends species, cell type, and growth characteristics and is exerted on all rapidly replicating cells, whether derived from normal or transformed tissues (4-7). The data reported here encompass more target cell lines and show that prolonged interaction with a majority of macrophages (effector to target cell ratio is 10:1) results in a distinct inhibition of target cell proliferation in every cell line examined, but the degree of cytostasis evoked by macrophages differs markedly from one cell line to another. The results were similar irrespective of whether ^3H -TDR or ^{125}I UDR was used as a pulse label to assess the residual proliferative capacity. Since macrophages do not replicate under tissue culture conditions and remain in the G_1 or G_0 phase of the cell cycle, incorporation of these labels is sharply restricted to target cells.

The data once again demonstrate that there are major differences in the susceptibility of cell lines. When a large array of cell lines is ranked by their susceptibility to macrophage-mediated cytostasis, normal replicating lymphoid cells (7), various virus-transformed lines (rat polyoma and B_{77} , mouse SV40), P-815 mouse mastocytoma cells, and some cell lines derived from human malignant tumors are especially sensitive; derivatives of the mouse L fibroblast, though consistently blocked after prolonged interaction with macrophages, showed large differences in their initial sensitivity not correlated with their degree of malignancy. Other lines such as 3T3, CHO, DMBA- and MCA-induced rat tumor cells, the lymphoblastoid cell lines, and recently explanted fibroblasts derived from normal adult or embryonic DA rat tissue, were resistant to macrophage-mediated cytostasis. Accordingly, it is evident that in rapidly replicating cell lines, factors other than capacity for *in vivo* malignancy or *in vitro* transformation determine susceptibility to macrophage-mediated cytostasis.

Earlier studies (4) showed that indicators of immune cytotoxicity such as those widely employed in lymphocyte-target studies (i.e., release of ^{51}Cr or uptake of trypan blue) were unsuitable for detection of macrophage-induced changes in target cell viability. Accordingly, in some experiments, the number of target cells remaining after various intervals of interaction with macrophages was counted for quantitation of cytotoxic macrophage effects on targets; the number of remaining targets shows some diminution by 24 hours and is further decreased as the interaction proceeds. However, many targets often remain in culture even after a 96-hour interaction with macrophages (table 4).

^{125}I UDR has been used more frequently as a label of target cells for the detection of lymphocyte-mediated

cytotoxicity (17-21). The main advantages of ^{125}I UDR are low spontaneous release and little, if any, reutilization. Accordingly, release of the isotope from prelabeled targets indicates cell death and lysis (22-25); since ^{125}I UDR is a gamma-emitting isotope, little preparation of samples is needed before counting. However, ^{125}I UDR is toxic (26, 27) and often blocks further cell replication. The present studies show, however, that despite considerable differences among the various cell lines, toxicity contributed by the labeling procedure can be held within acceptable limits, provided the isotope concentration is kept low and incubation is limited to 8 hours. Under such conditions, the inclusion of FUDR to increase preferentially the incorporation of IUDR into DNA in place of ^3H -TDR is a necessary precondition. With the use of these modifications, target cell lines were sufficiently labeled without gross signs of toxicity; i.e., ^{125}I UDR-labeled cells replicated at a rate similar to that of unlabeled controls. Thus the present work shows that this method of measuring cytotoxic (and cytostatic) capacities of effectors applies to targets long established in culture or recently derived from normal tissues, growing adherent or in suspension, or showing growth attributes typical of normal or transformed cells.

The accurate measurement of viability in a variety of targets and its application to macrophage-mediated effects are essential for furthering the understanding of the processes involved in the interaction between macrophages and other eukaryote cells. Our work clearly demonstrates that interaction of nonimmune macrophages with a variety of prelabeled targets is accompanied by a consistent release of the label. Such release is not usually detectable before 18 hours, but often increases as interaction proceeds. Beyond the principal demonstration of the existence of the potent cytotoxic or cytolytic capacities of nonimmune macrophages, the present findings, though still limited, are informative in many ways. Thus the data indicate distinct differences among the targets examined in their susceptibility to macrophage-mediated cytotoxic effects. For example, carcinogen-induced tumor cells and recent explants of normal fibroblasts, both of syngeneic origin, seem particularly resistant.

On the basis of morphologic grading of macrophage-mediated target cell cytotoxicity, neoplastic cells are uniquely susceptible as contrasted to their normal counterparts (3, 4, 10). However, both previous (6, 28, 29) and present findings showing that transformed cells are affected equally whether they are grown at permissive or nonpermissive temperatures, or whether they differ considerably in their malignant potential, now indicate that the prior conclusions were overgeneralized. These data and especially those about recent explants of rat fibroblasts indicate a quantitative rather than a qualitative difference in susceptibility of normal and transformed targets to macrophage-mediated cytotoxic effects. Further studies with cells recently derived from normal tissues (especially primary explants) are necessary to clarify this issue.

In many target cell lines, macrophage-mediated cytostatic and cytotoxic effects showed considerable parallelism. For example, recent explants of DA rat fibroblasts and carcinogen-induced DA rat cell lines, as well as human lymphoblastoid lines, were relatively resistant to both effects. However, in polyoma-induced rat and P-815 mouse cells, no such parallelism was observed; these cell lines were highly susceptible to cytostatic macrophage effects but resistant to cytotoxic macrophage effects. This discrepancy suggests that cytostatic and cytotoxic effects

are not necessarily closely related. Earlier works (5, 7, 30) have convincingly shown that the effects on cell proliferation, both enhancing and blocking, are mediated by soluble factors released from macrophages in *in vitro* culture. However, close contact with targets seems necessary for cytotoxic and/or cytolytic effects of macrophages (31).

Most of the present comparative studies have been performed in parallel with the same macrophage populations for all targets. This is important since comparisons of results of series of experiments have repeatedly disclosed large quantitative differences (6, 7). Such differences as previously observed in macrophage cytostatic activity were probably sometimes due to differing degrees of macrophage functional activities. Similarly, the present variability in cytostatic (table 1) and cytotoxic (table 5) macrophage-mediated effects is mainly due to variations in activity manifested by macrophages harvested from different groups of inbred DA rats. Other variables, e.g., target cell characteristics, did not significantly affect the outcome of the interaction. The quantitative differences encountered in the degree of macrophage-mediated cytostasis and cytotoxicity did not prejudice the principal conclusions discussed earlier.

Interpretation of the present findings is further complicated by the likelihood that most of the lines generally utilized as representative of "normal" cells, such as 3T3 or CHO cells, are not the unaltered host cells we seek. In this respect, recent findings demonstrating that inoculation of the "normal" BALB/c3T3 cell line attached to glass beads leads to malignant hemangioendotheliomas, are significant (32). Thus we have reasons for believing that the currently accepted view that postconfluence inhibition of cell division, low saturation density, and anchorage dependence are *in vitro* properties characteristic only of normal nonmalignant cells, should now be reassessed. These developments imply that eukaryotic cells once established as cell lines (i.e., no longer under host regulatory control) have the potential for malignancy. Accordingly, the findings obtained with established cell lines *in vitro* are not the direct and extrapolatable information as previously viewed.

Despite these ambiguities, one salient point does emerge. Among a larger number of transformed cell lines with considerably differing malignant potential, there is no correlation between the degree of transformation and the susceptibility to macrophage cytotoxic effects.

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